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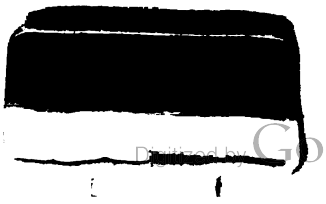
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THE INFLUENCE OF FEEDS UPON THE INTESTINAL BACTERIA OF CATTLE

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THE INFLUENCE OF FEEDS UPON THE INTESTINAL BACTERIA OF CATTLE

By

WILLIAM HARMON WRIGHT.

A Thesis Submitted for the Degree of

MASTER OF SCIENCE

UNIVERSITY OF WISCONSIN

1909

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The Influence of Feeds upon the Intestinal Bacteria of Cattle.

Part I.

Introduction.

After the microscope became useful in the study of bacteria, investigators applied it to all kinds of material. They soon recognized the fact that all kinds of fecal material are very rich in micro-organisms. Some of the earliest examinations were made directly on the feces of animals, in the endeavor to study processes of their nutrition. These examinations were mainly chemical. Nathnagel[1] 1881, Tappenier[2] 1884, Henneberg and Stohmann[3] 1885, Bahlman[4] 1886, and Kmeriem[5] 1886, were among the earlier investigators.

As early as 1880 all workers in bacteriology knew that bacteria were normally present in the digestive tract, of all common mammals, fowls, and fishes. Bienstock[6] in 1884 discussed the probable function of intestinal bacteria. In 1886 Sucksdorff[7] first plainly stated that the bacteria of the intestine probably had a function in digestion. He made quantitative examinations of human feces. He examined the feces passed by persons eating sterilized food. Also, feces from persons, whose

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food was not sterile, were examined. Such early investigations as this created an interest in the subject, and as bacteriological methods became more efficient different questions concerning intestinal flora were taken up and studied. Noteworthy among these was the work of Rovighi[8] in 1892, upon disinfection of the alimentary tract.

During the fifteen or twenty years preceding 1898 there had been more or less discussion concerning the meaning and importance of the intestinal bacteria. At this time Nutall and Thierfelder[9] came out with their classical piece of work, demonstrating that bacteria in the intestinal tract are not necessary at least, for the normal and healthy growth of guinea pigs. By means of the Caesarian operation the fully developed pigs were removed from the uterus and kept under perfectly sterile conditions, so that they could be fed and properly nourished. The operation was done so skillfully that the pigs grew and gained steadily in weight during all of the time the experiment was conducted. At the conclusion of the experiment the digestive tract of each experimental animal was found to be perfectly sterile.

Previous to 1898, Wutrich and Freundenreich[10] 1895,

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had studied the influence of different feeds upon the bacterial flora of the intestines of milch cows. Their experiments were with two cows over a short period of time. Reference will be made to their work subsequently. The main question has been as to just what part the bacteria of the intestine play in alimentary digestion. Only in the last decade has real interest been taken in this question. During this time bacteriologists and biological chemists of known repute have given their time to it.

As a result of the work of Lembke,[11] Kern,[12] Schattelins,[13] Hemmeter,[14] Kohlbrugge,[15] Metchnikoff,[16] Klein,[17] Matzuschita,[18] Rutmair,[19] Lewin,[20] Schmit[21] and Strasburger,[21] Gordon,[22] Houston,[23] Klimenko,[24] Bollner,[25] Ankersmit,[26] Mereskowisky,[27] and Andrews[28] the field as concerns man and the lower animals has been pretty well worked out. One exception to this exists. That is the herbivorous animals. Some work from the qualitative side has been done on them, but they have not been included by most investigators.

Present Status of the Question..

Since the time of Sucksdorff's[7] work in 1886, there has been a constant disagreement of authorities as to the meaning and importance of intestinal bacteria. In herbiv-

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orous animals, especially the ruminants, whose food consists mainly of crude fiber or cellulose, in the form of hay, corn-fodder, and various kinds of straw, there is found no specific digestive fluid for such substances as the celluloses. Even to the present time no intestinal secretion has been isolated from the digestive tract of any animal, which may be called a normal digestive fluid, and which will digest cellulose. Colin[29] claims that in all herbivorous animals except ruminants only two to four parts per hundred of the cellulose is digested in the stomach, while in the caecum and large intestine fifty to seventy parts per hundred disappears.

Investigators for the most part have experimented upon small omniverous or entirely carniverous animals, such as dogs, cats, rats, chickens and guinea pigs. The food of all of these animals, except it be the rat, is easily digested by the normal digestive juices. The food of dogs and cats is practically all protein and may be entirely digested by the proteolytic enzymes of the alimentary tract. Rabbits and guinea pigs live almost entirely upon green succulent foods as vegetables, grass, etc. This kind of food contains very little crude fiber and is easily broken up by the intestinal secretions.

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The question as to the need of the predominating intestinal bacteria, B. putrificus and the B. coli group of organisms, for an animal's nutrition has been the cause of some notable experiments. Nutall and Thierfelder[9] of whom reference has already been made, came to the conclusion that any animal may live normally without bacteria in its digestive tract. Schottelins[13] with his work upon chickens, came to the conclusion that they must have intestinal bacteria in order to live. He hatched chickens under sterile conditions, and gave them sterilized food, water and air for seventeen days. At the end of that time the chickens were weak and nearly dead. He then killed them and found the digestive tract of each one sterile. The controls hatched under the usual conditions grew normally. Schottelins[13] claimed that if he had not killed the chicks, they would have died. He concluded of course that the indisposition of the chicks was due to lack of bacteria in the digestive tract.

Madam Metchnikoff[16] undertook an experiment very much like that of Schottelins,[13] only she worked with the frog (Rana temporia). She sterilized the eggs, incubated them under sterile conditions in sterile water, and fed the young tadpoles sterile food. After a short time they died. Her conclusion was that the tadpoles died from the

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want of bacteria in their digestive tracts. On examination the digestive tracts were found to be free from bacteria.

Levin[30] in 1897 during the Nansen expedition in search of the North Pole, made numerous examinations of the air, water, and of the intestinal contents of animals, sea-fowl and fish in the arctic regions. His investigations showed the number of bacteria of any kind at 86°40' N. to be very small. In the examination of 1700 liters of air he found only eight bacteria developing. The water of the sea and glaciers gave only one colony in 11 c.c.. White bears, seals, white sharks, eider ducks, pinguins, black gulls, sea urchins, shrimps, etc., were taken, their intestines opened under aseptic precautions and the contents examined bacteriologically. Several of each different species were examined and in every instance, except one white bear and two sharks, the intestines were found sterile. All of the birds had sterile digestive tracts except one white gull. Stained preparations of the feces as well as the cultural methods employed failed to show the presence of bacteria. In the case of the white bear the organism found, culturally and morphologically resembled B. coli. All of these animals were in the best of condition in

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their native wild state when taken, and were living with sterile digestive tracts, except the three or four isolated cases.

These experiments stand out the most prominently on both sides of the question. Others as Sucksdorff,[7] Tissier, Ballner,[25] Hammerl,[32] and Lembke,[11] have shown that it is possible to reduce the number of normal intestinal bacteria from several millions per gram of feces to a few thousands, and still have the animal live normally on any variety of food.

The balance of evidence seems to point to the fact that some animals are enabled to live normally without bacteria in their digestive tracts. However, as to just how great an extent this is true of herbivorous animals, remains to be seen. Investigators have studied the putrefactive bacteria in man and the lower animals, and have found them in great abundance. Klein,[17] Matzuschita,[18] Schmit and Strasburger,[21] Mereskowisky,[27] Tissier,[31] Bienstock,[6] Lembke,[11] Raczynski[33] and Wrzosek[34] found B. coli and lactis aerogenes to be the predominating types in most animals. However, a majority of them claim that B. putrificus is very commonly present in large numbers. Bienstock,[6] in a review of the subject gives it as his opinion that these three species are the predominating ones

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in the intestine.

Numerous examinations of the intestinal tracts of herbivorous animals, from the chemical standpoint, have been made by Nothnagel,[1] Kmeriem,[5] Bohlman,[4] Lindsey and Holland,[35] Biel,[36] Rutmair,[19] Hoffmeister[37] and Holdfleiss.[38] The latter two investigators working independently and at different times, procured from the paunch contents of ruminants filtered, aqueous, solutions which were capable of digesting considerable quantities of cellulose. In the experiments of Holdfleiss,[38] the cellulose digested amounted to as much as forty to sixty per cent. Preparations of the mucosa of the same organ, the paunch, extracted and treated with phenol water gave no digestion of cellulose. This evidence points to the belief that no enzyme which digests cellulose is secreted by the digestive tract of herbivora, and that such an enzyme is produced by bacteria in the paunch and caecum.

The significance of the well known intestinal bacteria, (B. coli communis, and B. lactis aerogenes), in man at least and no doubt in most lower animals is apparently one of small importance from the standpoint of animal nutrition. The fact that they are there is perhaps more a matter of accident than one of necessity or of some happy provision of nature for the benefit of the

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animal.

Some very notable work was done by Klimenko[24] upon rabbits, dogs and guinea pigs. He fed the animals very large doses of bouillon cultures of non-pathogenic organisms such as B. prodigiosus and B. subtilis. By this means he succeeded in temporarily driving out the normal intestinal bacteria, and establishing a preponderance of the organism fed. This gave rise to intestinal catarrh and general indisposition. He concluded that any organism foreign to the intestine, whether pathogenic or not, if present in predominant numbers will disturb the animals general health. His reason for this is that they are not adapted to the environment of the intestine.

Strasburger[39] concludes that in man the predominant group of organisms (B. coli group) serves two purposes, neither of which are related to nutrition. These are (1) allowing only fermentation in the small intestine and not putrefaction as in the large; (2) inhibition of the growth of pathogenic organisms.

The fact that Omelianski[40] and Maze[41] have isolated three specific, cellulose digesting organisms from the feces of herbivorous animals, furnishes ground for the belief that the bacteria may be the means of cellulose

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digestion. The early work of Hofmeister[37] later confirmed by Holdfleiss[38] in 1896, seems to confirm this belief. Recently Ankersmit[26] in 1905, made a very extensive bacteriological examination of the digestive tracts of some fifteen normal, adult, healthy cattle. He found that the intestinal contents had marked cellulose fermenting power. However, he is not inclined to credit the digestion of fifty to seventy per cent of the cellulose to bacteria. He thinks that their action is too slow and they are not present in sufficient numbers. He suggests that this digestion may still be explained along the lines pursued by Hofmeister[37] and Holdfleiss.[38] In his work he was able to find B. coli present in every animal examined. From his figures it appears that not more than ten per cent of the total organisms present were B. coli. This percentage is not high enough for a predominant species. Although cellulose fermenting organisms were probably present there was no way to enumerate them. This being due to the fact that they are so difficult to cultivate in the laboratory.

The probable significance of yeasts and moulds has never been clearly explained and their importance is still a matter of conjecture. The difficulties encoun-

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tered in carrying on experimental fermentations with these different intestinal organsisms is that natural conditions cannot be duplicated in the laboratory. Differences in results due to change in composition of culture media always tends to give misleading results. The variable nature of the numbers and varieties of bacteria on the food is another important factor which may influence the results.

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Part II.

Experimental Work.

Reasons for undertaking the work - In May 1907 the Wisconsin Experiment Station purchased for experimental purposes sixteen head of grade Shorthorn, heifer calves. These were to be used for work in a feeding experiment. The plan as carried out was to divide the animals into four lots of four each. Four were to receive nothing but the wheat plant and its products. The third lot were to receive only the oat plant and its products, while the remaining four were to get a so-called "normal" ration of the three feeds mixed together.

During the autumn of 1908 it occurred to the members of the chemical department, that the bacterial flora of the intestinal tracts of these animals might be interesting. If this could be investigated and data procured it might throw some light upon the physiological investigations. With that idea in mind the present investigations were undertaken.

Method of Procedure - The different sides of the question were not easy to determine. It was questionable just what part of the activities of the intestinal flora of the animals was influenced by the different

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feeds. The method of examination was finally arranged as follows:-

(1) Make weekly examinations of each feces, having a standard set of conditions until the data gave either a positive or negative result.

(2) Determine the percentage of gram positive organisms in the fresh feces of the various groups.

(3) Bring out the organisms that grow best, under the given conditions, by inoculating mass cultures into flasks containing sufficient bouillon to allow all kinds of organisms to develop. By changing the fermentable substance in the medium the organism that was enabled to ferment it best would develop to the exclusion of others.

(4) Examinations were made by the regular methods for B. coli communis.

(5) Determine to what extent and how many strains of streptococci were present and study their fermenting powers.

(6) Find to what extent the different feces collected under sterile conditions could ferment cellulose.

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(7) Examine the feeds and see if there was any apparent relation between the flora found in them and in the feces.

(8) Study the milks from the animals and see if any bacteriological differences could be established.

The Quantitative Examination of Feces - The specimens were collected in sterile Petri dishes in the morning soon after being deficated. They were then taken to the laboratory and sown in lactose - litmus - agar plates and incubated at forty degress for forty-eight hours. The reaction of the medium was always one per cent acid according to the so-called Massachusetts scale. Each animal was known by a number, and the different numbers of the four groups were numbered as follows:-

Corn fed animals	Wheat fed animals
556	561
558	565
566	570
572	571
Oats fed animals	**Mixed fed animals**
557	554
567	555
568	562
569	563

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Before conducting the regular examinations for the quantitative data, it was deemed advisable to make a test of the method employed. A fresh sample of feces from heifer number 572 was selected. One gram of the sample was weighed as accurately as possible, under sterile conditions on a well adjusted trip scale. This scale was sensitive to ten milligrams. Dilutions of 1:10000, 1:100,000, and 1:1,000,000 were plated upon lactose-litmus-gelatine. As soon as the flask from which the plating was to be done was ready, three 1 c.c. portions were removed to the sterile Petri dishes while the contents were still in motion. Counts from the nine plates gave the following results:-

Table Showing Results of Test of the Method.

Dilution	Plate	No. bacteria per gram.
(1:10,000	1	6,500,000
(1:10,000	2	5,900,000
(1:10,000	3	6,100,000
(1:100,000	1	5,500,000
(1:100,000	2	6,000,000
(1:100,000	3	6,300,000
(1:1,000,000	1	4,000,000
(1:1,000,000	2	7,000,000
(1:1,000,000	3	8,000,000

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This test of the method employed showed as great accuracy as one can expect in any method at present used for quantitative bacteriological work. Two sets of plates were made each week when the samples of feces were collected. One, the agar plates, was incubated at 40 degrees C. for 48 hours. Another set made in lactose-litmus-gelatin was kept at room temperature which was something near 22 degress C. These plates were counted four days after plating. However, owing to changes in temperature of the room gelatin plates were often left as long as a week before counting.

In the tables given below in the left hand column will be found the numbers of the animals. Each table represents a group of animals showing the results of five consecutive weeks' examinations under constant conditions. Following the results for the four groups, with agar at 40 degrees C. will be found the results with the same groups and gelatin at 22 degrees C. The tables of averages follow the tables of weekly data.

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Number of Colonies at 40° C. From Corn Fed Animals.

No. of animal	1st week	2nd week	3rd week	4th week	5th week
556	8,000,000	2,200,000	3,500,000	3,330,000	2,400,000
558	1,200,000	5,300,000	4,300,000	4,220,000	4,310,000
566	9,000,000	1,700,000	2,100,000	1,030,000	3,040,000
572	2,100,000	2,320,000	8,000,000	8,150,000	9,240,000

Number of Colonies at 40° C. From Wheat Fed Animals.

No. of animal	1st week	2nd week	3rd week	4th week	5th week
561	1,240,000	1,100,000	1,540,000	1,300,000	5,230,000
565	396,000	400,000	4,300,000	4,000,000	4,300,000
570	1,240,000	900,000	700,000	2,100,000	4,200,000
571	2,340,000	1,800,000	2,120,000	3,000,000	6,330,000

Number of Colonies at 40° C. From Oats Fed Animals.

No. of animal	1st week	2nd week	3rd week	4th week	5th week
557	5,260,000	3,600,000	2,110,000	1,070,000	11,320,000
567	800,000	2,800,000	6,230,000	2,000,000	9,410,000
568	396,000	3,100,000	1,230,000	3,500,000	7,400,000
569	830,000	1,800,000	2,320,000	1,890,000	3,120,000

Number of Colonies at 40° C. From Mixed Fed Animals

No. of animal	1st week	2nd week	3rd week	4th week	5th week
554	6,100,000	4,200,000	3,030,000	5,400,000	1,200,000
555	1,930,000	800,000	7,010,000	4,200,000	3,240,000
562	1,200,000	7,000,000	4,300,000	6,000,000	780,000
563	11300,000	9,330,000	6,200,000	5,000,000	950,000

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Number of Colonies at 22° C. From Corn Fed Animals.

No. of animal	1st week	2nd week	3rd week	4th week	5th week
556	330,000	1,800,000	2,100,000	3,100,000	2,100,000
558	940,000	2,300,000	4,100,000	2,000,000	4,200,000
566	600,000	1,500,000	1,500,000	600,000	2,800,000
572	1,560,000	220,000	1,930,000	5,090,000	6,000,000

Number of Colonies at 22° C. From Oats Fed Animals.

No. of animal	1st week	2nd week	3rd week	4th week	5th week
557	4,100,000	2,800,000	2,300,000	,900,000	9,100,000
567	830,000	1,900,000	4,130,000	1,800,000	7,200,000
568	400,000	2,300,000	1,200,000	2,400,000	3,000,000
569	500,000	950,000	2,100,000	1,500,000	1,890,000

Number of Colonies at 22° C. From Wheat Fed Animals.

No. of animal	1st week	2nd week	3rd week	4th week	5th week
561	1,100,000	1,000,000	2,100,000	4,200,000	3,980,000
565	400,000	3,800,000	3,960,000	3,500,000	5,300,000
570	900,000	8,000,000	1,800,000	4,000,000	4,000,000
571	1,800,000	2,200,000	2,800,000	4,100,000	6,310,000

Number of Colonies at 22° C. From Mixed Feed Animals.

No. of animal	1st week	2nd week	3rd week	4th week	5th week
554	800,000	700,000	4,800,000	1,200,000	4,200,000
555	1,240,000	350,000	6,300,000	2,100,000	800,000
562	? 600,000	400,000	5,000,000	1,400,000	7,000,000
563	830,000	460,000	4,300,000	3,900,000	9,330,000

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Averages by Groups for the Five Weeks, Colonies at 40° C.

Group	1st week	2nd week	3rd week	4th week	5th week
Corn	5,260,000	2,880,000	4,475,000	4,182,000	4,747,000
Wheat	1,304,000	1,050,000	2,165,000	2,612,000	5,015,000
Oats	506,500	2,828,000	2,970,000	2,115,000	7,812,500
Mixed feed	5,132,500	6,843,000	5,635,000	5,150,000	1,542,500

From the preceding summary table a still more condensed table of averages may be made.

Group	Average for all Animals in Group for Five Weeks
Corn	4,308,800
Wheat	2,429,200
Oats	3,245,200
Mixed feed	4,860,600

These final figures if considered as absolute representatives of the results would immediately separate the groups into two classes, the corn and mixed feed animals being in a group with an average bacterial content of the feces of about four million bacteria per gram. However, this is not representative. For instance in the case of animal No. 563 of the corn lot, there is recorded for the first week a bacterial content per gram of 11,300,000. This is more than twice the average. Again in the same group during the fifth week animal No. 562 gave only

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780,000 organisms developing on the plates from one gram of feces. The same thing holds true for the animals of the wheat and oats groups. Where such wide differences exist from week to week averages cannot be taken as representative of the true relations between the groups.

Averages by Groups for the Five Weeks, Colonies at 22° C.

Group	1st week	2nd week	3rd week	4th week	5th week
Corn	623,000	1,866,000	2,656,000	3,396,000	3,775,000
Wheat	1,050,000	3,750,000	2,665,000	3,950,000	4,852,500
Oats	576,000	2,333,000	2,432,500	1,900,000	5,297,000
Mixed feed	743,000	477,500	5,100,000	2,466,000	6,843,000

The averages in the preceeding table presented about as great differences as did the agar plates at 40 degrees C.. Absolute figures for the results at 22 degrees C. gave a relation as follows:-

Group	Average for all Animals in Group for Five Weeks.
Corn	2,463,200
Wheat	3,253,500
Oats	2,495,700
Mixed feed	3,121,900

Absolute figures here show a very close average of all four groups. However, the corn and oats groups are

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nearly together. At the same time the results tend to throw the wheat and mixed feed groups together. If one examines the first table of averages, for example the oats group average for the first week, there will be found 576,000 colonies against the final average of 2,495,700. A difference too great for comparative results. Again take the extremes of individual animals. In the mixed feed group animal No. 562 gave 600,000 colonies the first week and 7,000,000 the 5th. Such extreme variations tend to give unreliable averages and they are of no value for comparative purposes.

Relation of Acid Forming Organisms to Feeds from the Quantitative Standpoint - The results of these examinations were procured from the number of acid colonies appearing upon the gelatin plates.

Acid Colonies Appearing from Corn Fed Animals.

No. of animal	1st week: per cent	2nd week: per cent	3rd week: per cent	4th week: per cent	5th week: per cent
556	65	25	30	70	10
558	30	15	25	42	15
566	12	10	40	30	48
572	18	12	60	50	50

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Acid Colonies Appearing From Wheat Fed Animals.

No. of animal	1st week per cent	2nd week per cent	3rd week per cent	4th week per cent	5th week per cent
561	27	16	20	55	32
565	47	24	12	20	36
570	31	18	3	38	18
571	15	12	40	40	44

Acid Colonies Appearing From Oats Fed Animals.

No. of animal	1st week per cent	2nd week per cent	3rd week per cent	4th week per cent	5th week per cent
557	10	5	55	15	39
567	28	25	38	25	40
568	50	30	24	32	60
569	20	18	30	41	42

Acid Colonies Appearing From Mixed Feed Animals.

No. of animal	1st week per cent	2nd week per cent	3rd week per cent	4th week per cent	5th week per cent
554	12	30	12	13	13
555	6	24	8	25	18
562	28	45	19	30	13
563	70	20	12	33	12

Averages of Acid Colonies of all the Groups.

Animals	1st week per cent	2nd week per cent	3rd week per cent	4th week per cent	5th week per cent
Corn	31	15	39	48	31
Wheat	24	17	24	38	37
Oats	24	24	38	28	45
Mixed feed	36	29	14	25	14

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Absolute figures for each group for the whole period of five weeks.

Group:	Per cent of acid colonies
Corn :	33 of all colonies on plates.
Wheat:	28 of all colonies on plates.
Oats :	31 of all colonies on plates.
Mixed feed:	23 of all colonies on plates.

A final comparison of the per cent of acid colonies developing upon the plates showed practically no difference between the groups, in so far as the number of acid colonies were concerned. These averages having been made from sets of results which vary in some instances from ten to forty-five per cent they can only be taken as roughly approximate. The nature of a part of the organisms forming these acid colonies can only be taken as a suggestion of what role fecal organisms may play in the different animals. This will be taken up subsequently.

The Relation of Liquefying Organisms to the Feeds from the Quantitative Standpoint - The data for this part of the work was procured from the gelatin plates. Just as soon as colonies were observed to be liquefying the medium a count was made. In some instances the

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number of liquefying colonies was so great as to make the total quantitative count of doubtful value. Such instances are noted by an interrogation point in the tables or the space left blank.

Number Of Liquefying Colonies From Corn Fed Animals.

No. of animal	1st week: per cent	2nd week: per cent	3rd week: per cent	4th week: per cent	5th week per cent
556	3	4	6	2	3
558	10	9	0 ?	0 ?	2
566	7	4	5	4	4
572	12	3	4	4	7

Number Of Liquefying Colonies From Wheat Fed Animals.

No. of animal	1st week: per cent	2nd week: per cent	3rd week: per cent	4th week: per cent	5th week: per cent
561	4	3	1	6	9
565	8	2	3	1	3
570	10	5	9	3	2
571	24	5	0	7	12

Number Of Liquefying Colonies From Oats Fed Animals.

No. of animal	1st week: per cent	2nd week: per cent	3rd week: per cent	4th week: per cent	5th week: per cent-
557	4	5	2	12	3
567	2	12	6	18	4
568	12	3	4	2	3
569	4	8	2	3	4

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Number Of Liquefying Colonies From Mixed Feed Animals.

No. of animal	1st week per cent	2nd week per cent	3rd week per cent	4th week per cent	5th week per cent
554	18	6	1	6	12
555	25	10	8	2	3
562	20	12	3	1	2
563	3	3	3	7	4

Average Percentage Of Liquefying Organisms By Groups.

Group	1st week per cent	2nd week per cent	3rd week per cent	4th week per cent	5th week per cent
Corn	8	5	5	3	4
Wheat	11	4	3	4	6
Oats	5	7	3	9	3
Mixed feed	16	8	4	4	5

Examination of the table of averages of the four groups of animals for five weeks showed no distinct quantitative difference which might be ascribed to the feeds. The percentage of liquefying organisms was low in all the groups. Much lower than is usually found in the feces of carnivorous animals. This was due in all probability to the feeds of all four of the groups not being of the same nature as the flesh foods of carnivorous animals. Also in the animals examined the food was not retained for the length of time it usually is in flesh eating animals.

A complete average of the percentage of liquefying organisms for each group for the five weeks shows -

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Group	Average Percentage of Liquefying Organisms
Corn	5
Wheat	5
Oats	5
Mixed feed	7

From the preceding table it may be seen that the average number of liquefying colonies did not exceed ten per cent of the total number on the plates. The lowest percentage was five per cent from the wheat lot. The percentages five, five, five and seven do not in themselves indicate any particular differences in numbers of liquefying organisms ascribable to the feeds of the respective groups. For instance in the mixed feed group where the percentage for the final average is ten, animal No. 562, of that group gave only one per cent of liquefying colonies for the fourth week. The same kind of variance is true of the wheat group where the final average of liquefying colonies is five per cent. Animal No. 571 of this group gave a count of the plates the first week with twenty-four per cent liquefiers. Where examinations gave such quantitative results as these it appeared that it was very difficult to attach any importance to them which could be due to the influence of the feeds.

At this point in the quantitative examinations it became apparent that there was no possible means of in-

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terpreting the data so as to ascribe the results to the influence of the various feeds. In fact there was a change quantitatively in the bacteria from the feces of each animal from day to day, and week to week, too great to allow of a valuable set of data.

The Influence Of The Nature Of The Culture Medium Upon The Quantitative Results - The results of the work of men who have studied intestinal bacteria has led them to employ different kinds of nutrient media for plate cultures. Matzuschita[18] used media prepared from extracts of different digestive organs. Gordon,[22] Houston,[23] and Andrews[28] made use of it with a high pepteon content. As high as five per cent and seven per cent peptone was used. This was found to be of advantage in bringing out fecal streptococci. As the quantitative plates were also employed for the qualitative work it was decided to us media in which the percentage of peptone was higher than usual. Media with five and seven per cent of peptone was employed. These changes in the medium were tried under both aerobic and anaerobic conditions. The results are shown in the following tables. They are averages of the results found in the original tables.

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Anaerobic medium at 22° C. on gelatin reaction + 1	efy- col- s at 22°C. one um robic	Liquefying colonies at 22° C. medium reaction + 1 anaerobic
	cent	per cent
780,000	7	0
500,000	4	0
970,000	3	0
550,000	9	0
Dish broken	?	Dish broken
800,000	4	2
1,100,000	7	9
800,000	3	10
830,000	0	5
990,000	2	8
960,000	1	12
1,540,000	0	4
600,000	0	1
1,130,000	6	2
440,000	3	0
670,000	4	0

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Table Showing Averages, By Groups, Of the Colonies

On Different Media.

Group	Agar 40°C. 7% peptone	Gelatin 22°C. 7% peptone	Agar 40°C 10% peptone.	Gelatin 22°C. 10% peptone	Agar 40°C. Reaction neutral to litmus.
Corn	9,450,000	4,625,000	1,460,000	1,107,500	4,075,000
Wheat	1,625,000	6,925,000	16200,000	10500,000	6,300,000
Oats	7,850,000	4,705,000	5,860,000	1,682,500	6,625,000
Mixed feed	13125,000	3,292,500	19625,000	9,630,000	4,475,000

Table Showing Averages Of Colonies By Groups

On Different Media.

Group	Gelatin 22°C. Reaction neutral to litmus	Anaerobic cultures Agar 40°C. 10% peptone.	Anaerobic cultures gelatin 22°C. 10% peptone	Anaerobic cultures agar reaction + 1. 40°C.	Anaerobic cultures gelatin reaction + 1 22°C.
Corn	3,222,500	882,500	842,500	790,750	700,000
Wheat	5,502,500	862,500	707,500	887,500	900,000
Oats	3,225,000	605,000	556,000	1,350,000	1,080,000
Mixed feed	3,825,000	860,000	817,500	805,000	2,460,000

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Table Showing Average Per Cents Of Acid Colonies On The Plates By Groups.

Agar 40° C.

Group	Acid Colonies on 7% peptone medium.	Acid Colonies on 10% peptone medium	Acid Colonies anaerobic culture 10% peptone.	Acid Colonies on medium reaction neutral to litmus	Acid Colonies medium reaction + 1 anaerobic.
Corn	30%	34%	32%	24%	90 to 100%
Wheat	27%	34%	37%	37%	22%
Oats	36%	19%	27%	over 90%	27%
Mixed feed	22%	17%	22%	36%	over 90%

Table Showing Average Per Cent Of Liquefying Colonies Upon Plates By Groups.

Gelatin 22° C.

Group	Liquefying colonies on 10% peptone medium	Liquefying colonies on 7% peptone medium	Liquefying colonies on medium neutral to litmus	Liquefying colonies on 10% peptone medium anaerobic culture	Liquefying colonies on media + 1 reaction Anaerobic culture
Corn	4%	9%	7%	6%	0
Wheat	4%	12%	7%	3%	7%
Oats	3%	5%	5%	2%	28%
Mixed feed	7%	5%	4%	7%	2%

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Table Showing Average Per Cents Of Acid Colonies On The Plates By Groups.

Agar 40° C.

Group	Acid Colonies on 7% peptone medium.	Acid Colonies on 10% peptone medium	Acid Colonies anaerobic culture 10% peptone.	Acid Colonies on medium reaction neutral to litmus	Acid Colonies medium reaction + 1 anaerobic.
Corn	30%	34%	32%	24%	90 to 100%
Wheat	27%	34%	37%	37%	22%
Oats	36%	19%	27%	over 90%	27%
Mixed feed	22%	17%	22%	36%	over 90%

Table Showing Average Per Cent Of Liquefying Colonies Upon Plates By Groups.

Gelatin 22° C.

Group	Liquefying colonies on 10% peptone medium	Liquefying colonies on 7% peptone medium	Liquefying colonies on medium neutral to litmus	Liquefying colonies on 10% peptone medium anaerobic culture	Liquefying colonies on media + 1 reaction Anaerobic culture
Corn	4%	9%	7%	6%	0
Wheat	4%	12%	7%	3%	7%
Oats	3%	5%	5%	2%	28%
Mixed feed	7%	5%	4%	7%	2%

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The averaged results in some instances when the nature of the culture medium was changed showed very appreciable changes, and differences. However, examination of the original figures showed that there were weeks and individual animals which gave results very different from the averages. In practically every instance the data procured showed such fluctuations that no quantitative separations based upon feeds could be made taking any one set of the different conditions employed as constant. One thing came up in the use of media with high peptone content which is notable. In practically every instance no matter what the reaction or conditions of the medium, the total count of colonies was higher than with the ordinary medium with the reaction plus one. This kind of medium seemed to favor a particular micrococcus which will be discussed subsequently.

In all of the various conditions tried for their effect upon the quantitative results, increase of peptone and anaerobic conditions seemed to be the greatest factor in influencing the liquefying organisms. The percentages of liquefying organisms in all of the groups were higher under aerobic conditions than under anaerobic conditions. Whether this result is due to lack of

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oxygen preventing growth of the liquefying organisms, or inhibition by the predominating anaerobe has not been determined.

The general tendency of the other types of colonies to show no localization into groups comparable with the feeds, was also evident in the percentages of acid colonies upon the plates where conditions were changed. In some instances a whole group of animals gave a set of plates which were entirely acid. This was true of the oats group. In each instance the same organism seemed to be the cause. In the case of the oats group the percentages of acid organisms were higher under all conditions observed than the other groups. The other groups gave results so variable that there cannot be said to have been any differentiation quantitatively among them.

The summary tables of results obtained under constant conditions and where temperature, reaction of medium, relation to oxygen, etc., have been observed seem to have indicated:

1. That practically no constancy in numbers of bacteria in the intestinal tracts of the different animals existed in relation to food.

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2. That very little constancy existed in numbers of bacteria in individual animals from week to week.

3. That the number of colonies appearing upon the plates depends upon the medium and conditions of growth.

Results Of Examination Of The Feces Of Cows Under Natural Conditions And Comparison With The Experimental Data - To find whether these experimental animals as a whole presented any difference in number and kind of intestinal bacteria four cows under typical batural conditions were studied. This examination was only quantitative.

Results Of Examination Of Cows Under Natural Condition.

Conditions under which cow was found.	No. of colonies on agar at 40° C. Reaction + 1	No. of colonies on gelatin at 22° C. reaction + 1	Acid colonies	Liquefying colonies.
Holstein U.W. herd	18,960,000	21,300,000	95%	2%
Jersey U.W. herd	35,000,000	32,000,000	100%	0
Family cow	15,000,000	12,000,000	75%	2%
Family cow	28,000,000	18,000,000	90%	5%

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Study of the results shows at once that the cows under normal conditions had many more intestinal organisms than the experimental ones. Also that the greater part of these were acid forming organisms. The percentage of liquefying colonies varied very little from that found in the experimental animals. By comparison it seemed that the experimental animals, all exhibited a changed flora. This was not so marked between the groups upon different feeds, but was shown in the great difference between all the groups and animals under natural conditions.

The so-called "normal" group of animals which received mixtures of the corn, wheat and oats plants were good controls upon the experiment. However, they did not represent natural conditions. The four cows under natural conditions received such substances in their food as beets, carrots, silage, alfalfa, and other kinds of hay. In the spring they received in addition pasture grass. This kind of a feed was a natural one.

The fact that three of the mixed feed group of animals aborted although not conclusive, points toward some kind of a physiological disturbance. The striking difference between the four cows under normal conditions and

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the whole number of sixteen animals of the experiment, indicates a very great change in the normal intestinal flora. This is evident in two ways (1) a much higher number of organisms per gram of feces, and (2) a very great number of acid producing bacteria. These differences appear to indicate that long continued feeding of these grains singly or together will greatly change the intestinal bacteria perhaps influencing digestion.

Results Of Examination Of The Feces By Means Of The Gram Method - The advantages of the gram method of staining were early recognized in the study of feces. Several workers have used it. Most notable in America perhaps is Dr. Herter. He has attached to it great value in studying feces for pathogenic bacteria, and for getting an idea of the nature of the fecal flora by direct examination. Its application to the feces of herbivora has been very little tried and results procured by direct examination have proved of little value. A suspension of about one gram of the feces was made in fifty or one hundred cc. of seven tenths per cent salt solution. After thorough shaking a drop was smeared upon the slide as evenly as possible and fixed, stained and examined directly. A very dilute solution of carbol

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fuchsin was applied just a few seconds to stain any organisms not stained by gram. Counts were made of the gram-positive organisms and the per cent obtained by comparison with the bacteria stained with fuchsin.

The inaccuracy of the method was obviously so great that little reliability could be placed upon it. The presence of considerable quantities of vegetable fiber and other indigested substances, some of which stained by gram and the larger part of which stained with fuchsin, gave an opportunity for error and rendered an accurate count practically impossible.

The best method that could be found to bring out the predominating organisms was that of Herter. It consisted of large flasks - 600 cc. - with a bouillon medium prepared with different kinds of fermentable substances. To about three hundred cc. of the sterile medium in a flask about one gram of each feces was added. The flasks were placed in the incubator for twenty-four hours and then their contents examined by the Gram method.

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Percentages of Gram Positive Organisms in Feces According to Groups.

Group	No. of animal	Per cent of Gram positive organisms by direct exam. of feces.	Per cent of Gram positive organisms in 24 hours old bouillon cultures
Corn	556	7%	40%
	558	5%	75%
	566	18%	90%
	572	50%	90%
Wheat	561	15%	95%
	565	30%	85%
	570	18%	85%
	571	12%	96%
Oats	557	11%	91%
	567	15%	83%
	568	12%	92%
	569	18%	87%
Mixed feed	554	3%	95%
	555	4%	90%
	562	7%	94%
	563	15%	96%

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Averages by Groups of the Per Cents of Gram Positive Organisms.

Group:	Gram positive organisms after 24 hours per cent	Gram positive organism by direct examination per cent.
Corn :	74	20
Wheat:	90	19
Oats :	88	14
Mixed feed:	94	7

The greatest number of gram positive bacteria by direct examination was found in the feces of the corn animals. The next lowest was the wheat, then the oats and last the mixed feed animals. Apparently this relation was not a lasting one as the examination of the bouillon cultures showed a new relation. However, all of the feces showed a marked increase in the medium used. The results from the bouillon cultures showed the predominating Gram positive organisms to be relatively higher than appeared in the direct examination. The results for the wheat animals 90%, oats animals 88%, and mixed feed animals 94% are very much the same. They are too near the same to allow of any conclusions as to a possible difference in the per cent of Gram positive

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organisms being caused by the different feeds.

The Predominating Organisms From Bouillon Cultures. The Gram positive organisms in the bouillon cultures after twenty-four hours were usually of several different types. As time went on and the flasks remained at room temperature the persistence of one or more organisms was observed and the disappearance of others. This apparently was due to optimum conditions being present for the predominant organisms. By using lactose and dextrose bouillon two kinds of organisms were given a chance to develop. The organisms which usually predominated in the dextrose medium were those which produced quantities of gas. Those in the lactose medium sometimes produced gas, other times no gas production was observed. The final predominance of one or more organisms was complete at the end of a week. Apparently the temperature of incubation made no difference.

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Table Showing The Predominating Organisms From The Corn Fed Animals.

No. of animal	Lactose Bouillon 40°C. 1day	3days	5days	7days	Dextrose Bouillon 40°C. 1day	3days	5days	7days
556	B.	B.	B.	B.	B.Y.	Y.	Y.	Y.
558	B.S.	B.S.	S.	S.	B.	B.Y.	Y.	Y.
566	B.	B.	B.Y.	Y.	B.Y.	Y.	Y.	Y.
572	B.	B.	B.Y.	Y.	Y.	Y.	Y.	Y.

Table Showing The Predominating Organisms From The Oats Fed Animals.

No. of animal	Lactose at 40°C. 1day	3days	5days	7days	Dextrose at 40°C. 1day	3days	5days	7days
557	S.B.	S.	S.	S.	S.B.	S.Y.	S.B.	S.B.
567	B.S.	B.S.	B.S.	B.S.	B.S.	S.Y.	Y.	Y.
568	B.	B.	B.	B.	B.B.	S.Y.	Y.	Y.
569	S.	S.Y.	S.Y.	S.Y.	Y.S.	Y.	Y.	Y.

B. = Bacilli: S. = Streptococci: Y. = Yeast.

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Table Showing The Predominating Organisms From The Wheat Fed Animals.

No. of animal	Lactose at 40° C. 1day	3days	5days	7days	Dextrose at 40° C. 1day	3days	5days	7days
561	S.	S.	S.	S.	S.	S.	S.	S.
565	B.	B.Y.	Y.	Y.	B.Y.	Y.	Y.	Y.
570	B.S.	B.Y.	Y.	Y.	B.Y.	B.Y.	Y.	Y.
571	B.Y.	Y.	Y.	Y.	B.Y.	Y.	Y.	Y.

Table Showing The Predominating Organisms From The Mixed Feed Animals.

No. of animal	Lactose at 40° C. 1day	3days	5days	7days	Dextrose at 40° C. 1day	3days	5days	7days
554	B.	B.	B.	B.	B.Y.	B.Y.	Y.	Y.
555	B.	B.Y.	Y.	Y.	B.Y.	Y.	Y.	Y.
562	B.	B.	B.	B.	B.	B.Y.	Y.	Y.
563	B.S.	B.S.	S.	S.	B.S.	B.S.	S.	S.

B. = Bacilli: S. = Streptococci: Y. = Yeast.

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Usually after about three days the predominant organism became prevalent and could be asily detected by microscopic examination of the contents of the flasks. In nearly every instance either a yeast or some form of streptococcus was the predominant organism. In the results that were obtained the yeast was found in the feces of all of the animals except three. Repeated examination showed it to also be present in these. According to groups there appeared to be very little difference in the organisms according to the methods employed. In the cases where the bacilli were persistant the organisms resembled B. coli in form and mode of growth. The yeast was always from the characters studied the same species. It appeared to be the most often present in the wheat fed animals and the most easily found there. The particular streptococci found were most plentiful in the feces of the wheat and oats fed groups. The significance of these two classes of organisms was only found by special study after isolation. Each one will be taken up in detail subsequently.

Examinations for B. coli Communis - When the plates were examined for quantitative purposes any significant colonies which appeared to be members of the B. coli

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group of organisms were isolated and studied. Thirty eight such examinations were made, and each feces studied. Complete routine examinations were not made, but enough results were procured to warrant the conclusions.

Table Showing Results of Tests For B. Coli Communis.

Property studied	No. of cultures examined	Positive	Negative
Motility	15	12	3
Gas in lactose-agar shake cultures	38	35	3
Acid and gas in dextrose peptone	38	30	8
Indol in broth (Nitrite H_2SO_4 Test)	38	25	13
Litmus milk, acid and clot	38	24	13
Nitrates reduced to nitrites	38	34	4
No liquefaction of gelatin	38	30	8
Acid and gas in lactose peptone	38	27	11

The results of the tests as recommended by Houston[23] for the identification of B. coli seem to indicate that it was the organism worked with. However, the results are not conclusive. Of all the tests made with the different properties of the organism studied, seventy-seven per cent were positive for B. coli. Gas formation was observed each time feces were plated. The gas formula $\frac{H}{CO_2}$ ranged from $\frac{1}{2}$, through $\frac{1}{1}$ to $\frac{2}{1}$ or $\frac{3}{1}$. Where a small amount of feces was added directly to the fermentation tube varying gas formula always resulted.

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All of the results of the methods employed indicated the presence of B. coli. But the results did not show that the colon group of organisms are predominant in the digestive tract. Many observers have claimed that the intestinal bacteria of practically all kinds of animals are principally B. coli and allied organisms. This has not been found to be the case in the animals examined.

The Significance Of The Streptococci In The Feces - Up to the present time there has been a great deal of importance placed upon the bacteriological examination of water supplies. This has led to many different methods of examination. As the most important and most common pollution of water supplies, has been from the excreta of men and animals, the bacteria always associated with such pollution have been sought after in the examinations. The ordinary routine methods have been used almost exclusively up to the present time. In 1903, Gordon[22] of England advanced the idea that the streptococci from human feces possessed different fermenting powers than those from other sources. As streptococci are always present in polluted waters it seemed a probable means of determining whether the pollution came from man or animals when found in a water supply. Later

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work by Houston[23] in 1903-04 seemed to confirm this. He also developed the idea that streptococci possessed characteristic fermentation tests upon carbohydrates of varying degrees of fermentability.

Fermentation Power Of Streptococci Isolated From Feces.

No. of organism.	Agar Slope.	Gelatin stab.	Plain bouillon.	Litmus milk	Acidity in Milk Per cent 1day	3days	7days	Lactose	Glucose	Raffinose	Xylose	Arabinose	Inulin	Conferin	Mannite	Starch
1	Scant	-+	Turbid	Acid: slow: clot:	.18	.78	.75	+	+	+	+	+	-	-	+	-
2	Scant	-	Turbid	No ?: cng.:	.22	.66	.70	+	+	-	+	+	-	+	-	-
3	Scant: transp	-	ppt.	Slow: acid: no clot:	.15	.66	.73	+	+	-	+	+	-	+	-	-
4.	Pro-fuse	-	ppt.	Slow: acid: no clot:	.20	.29	.40	+	+	-	+	+	-	+	+	-
5	Scant	-	ppt.	acid: clot: 7days	.24	.59	.62	+	+	-	+	+	-	-	-	-
6	Scant	-	pelli-+cle	acid: clot: 1day:	.25	.40 +	.52	+	+	+	+	+	-	-	-	-

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The different strains of streptococci examined were not classified according to grouping of animals on account of the fact that several of the different cultures isolated came from the same group of animals, and were found in many instances to be the same one. Those showing only by their fermenting powers that they were different were taken. Some of them from their properties would seem to indicate that they are strains of the same organism. However, there was always one or more marked differences to show that each was distinct. All of them fermented lactose, glucose, xylose and arabinose. Three fermented coniferin and three did not. Two fermented mannite. With these facts we might have two classes of the six organisms. Three in a class fermenting coniferin. These were numbers two, three and four. The remaining three came into the other class. However, in the first group one (No. 1) fermented mannite. Also number four in the second fermented it. This made a difference in each group among the members.

Houston[23] in a summary of the fermentation tests of fecal streptococci says, "arrangement from bouillon cultures is short chains; in broth uniform turbidity; acid and clot within five days at 37° C. in litmus milk. All

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were non-pathogenic for mice." The six strains of organisms studied were all growing in short to medium length chains. Only two produced uniform turbidity in broth cultures. Three did not produce any clot, but the milk became acid in about a week. One clotted it in twenty-four hours and one in a week. These showed a marked difference from the results of Houston[23]. There were not enough strains, of different characteristics, isolated to get a final result. The organisms studied were not held long enough to study their fermenting powers. Whether they actually differed from human streptococci, was not possible to ascertain from the data procured. Twort[45] in his preliminary work 1908, came to the conclusion that intestinal bacteria are too variable in their properties and lose their initial powers too easily for a determination of cultural characteristics.

The Significance Of Yeasts In The Feces - The studies of the predominating organisms, which has been discussed, showed the presence of a yeast in each of the sixteen animals' intestinal tracts. This organism when placed in the proper conditions showed marked activity. Its form and arrangement resembled very closely the Pastorianus series of yeasts described by Hansen[42].

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Since it has been found in all of the animal's feces it is difficult to ascribe its presence to any of the feeds. Cultures isolated from each of the sixteen feces gave the following results with no deviations.

All the cultures	Litmus milk.	Dextrose	Sucrose	Lactose	Raffinose	Xylose	Arabinose	Inulin	Coniferin	Mannite	Starch	Gelatin
	-	+	+	-	-	+	+	+	-	+	-	-

Every culture of the organism was the same in form, arrangement, staining properties and growth. It did not appear from the results obtained that the organism alone was able to break down substances of very complicated chemical composition. From these facts it appeared doubtful if it could have any very important part to play in the nutrition of the animals. However, it is possible that it may play some part in the digestion of the cellulose and crude fiber substances. In the fermentation tests it fermented xylose and arabinose, products of xylans and arabans which are found in the food of herbivorous animals to a great extent. It is very probable that the fermentation of these substances is an aid in digesting them. In the examination nothing developed

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Strips of Filter Paper Partly Digested by the Fecal Organisms.

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which seemed to show that the yeast was in any way influenced by or a product of the methods of feeding and the nature of the food.

The Fermentation Of Cellulose - Since cellulose digestion is very important in the nutrition of ruminants some experiments were made with the idea of finding if the feces had any digesting power which might be the result of bacterial action. As to just how cellulose digestion takes place is still an undecided question. The work of Omelianski[40] and of Maze[41] has demonstrated the existance of specific cellulose fermenting organisms. As the former investigator procured his organisms from horse feces it seemed very probable that the animals under study would give similar results.

Specimens of the feces collected under sterile conditions were examined. They were taken to the laboratory as soon as collected and about one gram of each specimen added to each of sixteen thirty c.c. test tubes containing Omelianski's medium. This was composed of,-

K_2HPO_4	1 g.
$MgSO_4$	.5 g.
$(NH_4)_2 SO_4$	1 g.
NaCl	Trace
$CaCO_3$	Added often enough to neutralize acid.
H_2O	1000 c.c.

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To the tubes containing the medium and the feces was added strips of Swedish filter paper about seven centimeters long and one centimeter wide. All sixteen test tubes were then plugged with rubber stoppers having two holes and the whole set connected in series by glass tubes bent in a "U" shape. A delivery tube reached from the last tube into a tube of mercury. The mercury acted as a release valve for any gases that collected. The whole apparatus was then set in the incubator and kept at 40° C. After seventy-two hours four of the tubes showed vigorous digestion of the filter paper.

Table Showing Cellulose Fermentation By Corn Fed Animals.

No. of animal	Date of inoculation of tube	First sign of fermentation days	Fermentation half completed days	Fermentation completed days
556	8 a.m.	3	4	8
558	8 a.m.	6	8	14
566	8 a,m,	4	6	10
572	8 a.m.	3	6	18

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Table Showing Cellulose Fermentation By Wheat Fed Animals.

No. of animal	Date of inoculation of tube	First sign of fermentation days	Fermentation half completed days	Fermentation completed. days
561	8 a.m.	3	4	7
565	8 a.m.	6	10	10
570	8 a.m.	4	10	16
571	8 a.m.	3	6	13

Table Showing Cellulose Fermentation By Oats Fed Animals.

No. of animal	Date of inoculation of tube	First sign of fermentation days	Fermentation half completed days	Fermentation completed. days
557	8 a.m.	4	6	12
567	8 a.m.	6	10	6
568	8 a.m.	9	16	20
569	8 a.m.	9	10	39

Table Showing Cellulose Fermentation By Mixed Feed Animals.

No. of animal	Date of inoculation of tube	First sign of fermentation days	Fermentation half completed days	Fermentation completed days
554	8 a.m.	5	10	23
555	8 a.m.	3	4	9
562	8 a.m.	11	17	33
563	8 a.m.	4	10	26

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Averages of the time of fermentation beginning and the completion shows,-

Table Showing Average Time Of Fermentation By Groups.

Group:	Average time of beginning fermentation:	Average time of ending fermentation
Corn :	4 days	10 days
Wheat:	4-1/2 days	14 days
Oats :	7 days	20 days
Mixed feed:	6 days	22 days

These results showed two things, (1) all the feces possessed a strong power of digesting cellulose, (2) this power was manifest in a very short time. All previous investigations showed that no fermentation had ever been observed to take place earlier than five days. In the case of the animals examined the members of the corn and wheat groups averaged four and one-fourth days as the time of starting. However, two of the tubes (numbers 565 and 556 showed vigorous digestion in seventy-two hours). In about one week to ten days the fermentations usually stopped, due to an excess of organic acids being produced. Upon the addition of $CaCO_3$ they became active again. The significance of these cellulose fermenting organisms in ruminant nutrition has not been fully demonstrated. The fact that they are there, and that they

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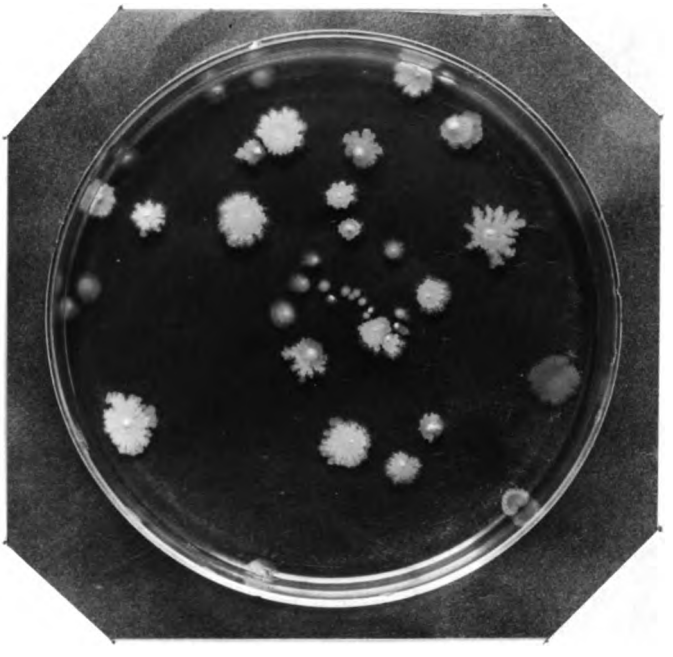
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can produce digestion of filter paper in a mineral solution in practically three days is very significant. The apparent greater activity of the organisms in the feces from the corn and wheat animals, seems to show that the nature of those grains as food substances induces greater activity upon the part of the bacteria. But different feces in the same group fermented cellulose so differently that it is very possible further investigation would show that the activity of the culture depends upon getting a uniform sample of feces. The amount of feces taken for the inoculation is also an important factor.

Moulds Present In The Feces Collected Under Sterile Conditions - Plates made with the lowest dilutions, $\frac{1}{10000}$ and $\frac{1}{100000}$ always contained colonies of moulds in about one week at room temperature. Examination of the feeds showed an abundance of moulds, but they were usually of a different character from those appearing in the feces. The high result of 500,000 colonies of Actinomyces odorata, being shown by the feces plates when examination of the feeds indicated none, was difficult to understand. All of the feces yielded A. odorata as well as various forms of penicillium and mucor. Whether the moulds grow enough in the intestine to produce fruiting bodies is

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Colonies of the Yeast from Feces, on Dextrose Agar.

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doubtful. According to Boycott[43] this might be possible as his observations seemed to indicate the presence of sufficient oxygen in the intestines for mould growth. In passing through the animal, if there was a great number of mould spores upon the feed, they might only partly germinate and the remainder pass out in the feces and then appear as colonies upon the plates. Hansen[42] favors this view of it.

The results of the examination developed the fact that the flora of the feeds and not the physiological condition of the animals was related to the moulds in the feces. As this brought out an examination of the feeds the relations of the moulds to the feeds will be discussed subsequently.

The Organisms Present Upon The Feeds Collected Under Sterile Conditions - The presence of a specific organism in the feed of an animal, if in excessive numbers, has been found to appear in the feces. Work done by Klimenko[24] and Wutrich and Freundenreich,[10] showed this to be the case.

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Table Showing Results Of Freundenreich and Wutrich.

Feed	Cow No. 1 No. bacteria per gram feces	Cow No. 2 No. bacteria per gram feces
Grass alone	11,125,000	2,900,000
Hay alone	300,000,000	100,000,000
Hay & sour potatoes	18,000,000	13,000,000
Brewery mash	19,375,000	13,375,000

Two milch cows were fed upon grass alone, hay alone, hay and sour potatoes and brewery mash with the results as shown in the preceding table. There can be no doubt that the feeds in these instances did influence the flora of the feces to a marked extent. The kinds of organisms found bore a direct relation to the feed. This was true especially when the cows received hay. Fully one third of the organisms found in the feces were bacillus subtilis.

All of these feeds were selected as extreme types, and were carriers of special types of organisms in predominance. Such a condition confirms the work of Klimenko[24] where the flora of the feed is established in the intestine. All observations were made when the particular feed was given for only a short time. The animals for a longer time might have been adapted to the conditions and shown no very great differences.

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In animals which were used in the work given in this paper conditions were different from those of other investigators. The animals were getting feeds very much alike in physical composition, origin, and degree of moisture. The examination of the feeds of the four groups did not reveal many differences which were constant for each separate group. The greatest difference was in the number of moulds upon the plates from the corn and mixed feed groups as compared to the oats and wheat groups. They were the same kinds in general except that the number upon the plates from the corn and mixed feed groups were very much greater than from the oats and wheat groups.

The Bacteria Present In Milk Collected Under Sterile Conditions - The importance of the influence of feces bacteria in the dairy industries is recognized by all dairy and creamery owners. They are the constant foe of the butter maker and cheese maker when even a very few of them are present in milk or cream. This together with the idea that such a long period of living upon abnormal feeds might have produced changes in the normal udder flora, were the principal reasons for study of the milks.

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Due to the fact that part of the animals were not giving milk at the time of the examinations, results are not complete. The method was so planned that only the representative udder flora could be studied. A number of 600 c.c. Erlenmeyer flasks were plugged with cotton and sterilized. These were taken to the barn at milking time and after thorough cleaning of the flanks and udders of the cows and the milkers' hands, were nearly filled with milk by milking directly into them. Only the last milk was taken, the first portion being allowed to cleanse the teats as thoroughly as possible.

The samples procured in the foregoing manner were immediately taken to the laboratory and placed upon ice. When cool they were plated upon lactose agar and incubated at 40° for twenty-four hours.

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Table Showing The Results Of The Work On Milk From Corn Group.

No. of animal	No. of colonies per lcc. of milk	Nature of organisms present	Nature of a curd of the milk one week old	Per cent of acid 1 week
556	300	White coccus	Gasy settled out	0.78
558	450	White and orange cocci	Curd very gasy	0.76
566	500	White cocci	Clear layer separated out	0.62
572	300	White cocci	Gasy curd separated out	0.81

Table Showing The Results Of The Work On Milk From Wheat Group.

No. of animal	No. of colonies per lcc. of milk	Nature of organisms present	Nature of a curd of the milk one week old	Per cent of acid 1 week
561	150	White coccus	Solid curd	0.76
565	200	Yellow cocci	Solid curd	0.62
570	150	White coccus small bacillus	Clear layer separated out	0.71
571	300	Yellow cocci	Solid curd	0.80

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Table Showing Results Of The Work On Milk

From Oats Group.

No. of animal	No. of colonies per lcc. of milk	Nature of organisms present.	Nature of a curd of the milk one week old.	Per cent of acid one week
557	400	Orange coccus	Gasy curd	0.72
567	--	No sample procured		--
568	150	White coccus	Clear layer separated out	0.65
569	200	White coccus and bacilli	Clear layer separated out	0.86

Table Showing Results Of The Work On Milk

From Mixed Feed Group.

No. of animal	No. of colonies per lcc. of milk	Nature of organisms present	Nature of a curd of the milk one week old	Per cent of acid one week
554	400	White and yellow cocci	Clear layer solid curd	0.54
555	200	Yellow cocci	Clear layer solid curd	0.42
562	400	White cocci	Clear layer solid curd	0.74
563	350	Yellow and orange cocci	Clear layer solid curd	0.97

The nature of the colonies developing upon the plates and the number of them indicated the presence of the usual udder organisms. No change or differences from ordinary animals or between groups could be noticed.

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Chemical and physical examination of the milks showed them to be very much changed in composition. It was thought that such changes in composition might change the normal udder flora. However, such was not found to be the case. Likewise the acidity in one week's time was nearly the same for all four groups.

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The Relation Of The Results To The Physiological Condition Of The Animals.

Part III.

The sixteen heifers were in average condition when purchased. They were immediately given the four different kinds of foods with no changes. In a short time the corn and mixed feed animals began to gain in weight and continued growth. The wheat and oats fed animals gained very slowly and in the course of a year appeared stunted and deprived of natural development. The consumption of water and salt varied in the different groups apparently with the progress of the animal's poor nutrition.

The corn fed animals, in particular gained constantly and grew to normal adult development. Their appearance was always good, hair smooth and skin loose. The wheat and oats fed animals,especially the former,had a rough, shaggy, appearance. They were "hide bound" and skinny. Examination by feeling seemed to indicate a hardened condition of the tissues under the skin. The oats fed animals also exhibited the same characters more or less. They also had failed to gain constantly in weight. The mixed feed animals gained and kept a good

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appearance, but apparently did not do so well as the corn fed animals.

Before the time for parturition three of the animals had aborted. One in the mixed feed lot and two of the wheat animals. The abortions from the wheat lot took place about a month early. This strain upon what reserve energy the wheat animals had placed,them in a serious condition. Only by the use of tonics is the feed in the form of mineral salts were they placed upon the road to recovery. The calves of the wheat and oats animals were very weak when born. All of them were unable to stand for several days. Such a weakened and debilitated condition of the mothers and correspondingly weak calves indicates a profound disturbance due to the long feeding of one kind of substance.

The mix feed and corn animals passed through the normal parturition period except one mixed feed one which aborted early. Calves from the mixed feed animals were unfortunately poorly handled and none lived. Those from the corn fed animals stood up very soon after being born. They were active and alert and remained so. The mothers stood the parturition period very well and maintained a normal weight. Apparently the corn was very nearly ful-

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filling all of the requirements for normal body activities.

The milk produced by the animals of the various groups varied widely in quality and quantity. The wheat and oats animals gave a poor, thin watery like product apparently deprived of some of the regular amounts of milk constituents. The quality was good and the yield from the corn animals especially was very great, for young animals of a poor dairy breed. Some of them gave as high as thirty pounds of very good milk per day.

The general differences in the four groups, which indicated in the case of the corn fed animals apparently optimum conditions for normal metabolism, were the opposite in the wheat and oats groups. Apparently a minimum in the wheat group for normal metabolism These differences were marked and obviously due in some way to the influence of the feeds. The bacteriological examinations did not develop specific group differences which could be the cause of such profound changes in the condition of the animals. Not a single parallel difference in any one phase of the bacteriological investigations can be found comparable.with any such difference as poor milk yield, debilitated condition of the animals,

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and weak calves. Subsequent investigations might show the influence of different types of intestinal bacteria which could give the effects noticed. However, the present work has failed to connect the physiological changes with special bacterial phenomena.

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Conclusions.

Part IV.

1. The quantitative examination of feces is accurate only within wide limits. Time, medium upon which the samples are plated, reaction of the medium and the temperature at which the plates are incubated are all factors which influence results.

2. The number of organisms in the feces of a given animal, will vary in short intervals of time sufficient to make the results of little value.

3. Feeds that are nearly alike in physical texture, degree of moisture and age as preparations of corn, wheat, oats and mixtures of these grains, will not apparently change the bacterial content of the feces so that the variations will show above the influence of such factors, as medium, period of incubation, reaction of medium, temperature, etc.

4. The fact that cellulose fermenting bacteria are present in the feces in sufficient numbers to cause vigorous fermentation of filter paper in three days is very significant and will bear further investigation.

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5. The reactions of the different strains of streptococci isolated gave results different from those indicated by other investigators for streptococci from human feces. However, not enough strains of streptococci were isolated and those that were isolared, were not studied thoroughly enough to sttach importance to the fermentation tests for work in sanitary water analysis.

6. The presence of mould spores was probably incidental and related to the number of them upon the feeds.

7. Apparently the predominant species of bacteria in cattle at least is not B. coli communis. Streptococci and staphlococci were found to be in the greatest numbers.

8. Each of the sixteen animals used in the experiment did not have normal intestinal flora. This was shown by the lower numbers of organisms as compared to normal animals.

9. The results of the application of the methods selected have failed to connect the important physiological changes with bacterial phenomena.

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APPROVED E. G. Hastings

Asst. Prof. Ag. Bacteriology

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